NOTES ON POLYCHROMATIC PHOTO-MICROGRAPHY

BY

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The value of photography as applied to biological research has increased markedly during the last decade by reason of the perfection of technique in the manufacture of the gelatinobromide dry plate, rendering easily possible results which were previously accomplished with great difficulty, if at all.

This is most evident in photomicrography, in which, using the product of the highest skill of the optician would not suffice to produce the required results, without the aid of the color sensitive plate. By the combination of plates sensitive to color with objectives and eyepieces, either achromatic or apochromatic, it is possible, when properly illuminating the object, to produce photomicrographs in monochrome, of the highest excellence. In many cases it would be quite unnecessary to seek to improve on such results, the monochromatic rendering of the various stains of the specimen conveying to the observer a correct impression of the object; but there are some specimens which have a special value due to their color contrast, and while isochromatic photomicrography can render these in monochrome, there is often a desire, as evinced in some of our text-books by colored illustrations, to add the value of color differences to those of form, light, and shade. Such colored illustrations are, however, open to the objection that they are products of the direct handwork of man, being either lithographs or separate block prints, in color, and frequently in addition being reproduced from a colored sketch or hand

drawing. If these objects could be as well rendered by photomicrography, and printed in color by mechanical means, without the intervention of handwork other than that pertaining to such presswork as involves the application of color by roller to a printing plate, we should have a colored object, rendered both as to its form and color by photomicrography, and hence free from the personal-equation error common in varying degree to all freehand drawings and reproductions of microscopic objects.

While the reproductions of gross objects in color by the aid of photography and mechanical color printing has been accomplished commercially for a number of years, the reproduction of objects, in color, by the aid of photomicrography has not been successful, some even asserting that it could not and probably never would be done, because the images of an object, as projected by the different colors of the spectrum, were of different sizes, and hence never could be made to register by any practical method. This is an interesting and plausible theory, but, nevertheless, an incorrect one, for I have found that, with adequate apparatus and exercising due care, the production of polychromatic photomicrographs is quite possible and, to one accustomed to working in photomicrography, not exceptionally difficult.

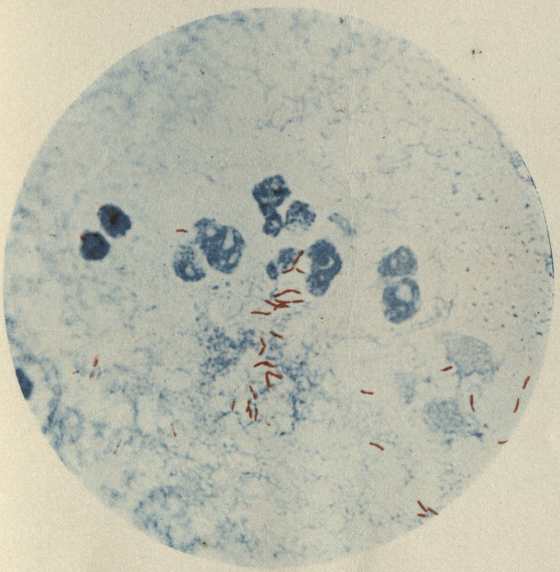
The initial experiments were made during the winter of 1895–'96 with old achromatic objectives giving a magnification of from 30 to 190 diameters, the results having since been shown at the Academy of Sciences, New York; the Pathological Society, New York; and the Royal Photographic Society, London. The three-color process of M. Ducos Du Hauron, as used by Mr. Edward Bierstadt, was chosen on account of the accuracy of the results in ordinary photography. The later experiments were on the same general lines as the first, but were directed specially to obtaining results at high magnifications, and for that purpose a Zeiss 2 mm apochromatic oil immersion objective was employed, the achromatic condenser being at the same time connected with the back of the slide by immersion oil, so that the full aperture of the objective was utilized.

I use as the source of light the electric arc, the feeding mechanism of which is controlled by a shunt coil, so that the carbons feed together every few seconds; the crater is thus maintained in the centre of the field until the carbons are consumed. The light is modified by diaphragms and sometimes by ground glass, and is then passed through the color screen to the condenser, and thence through object, microscope, and camera, to the sensitive plate. The color screens are collodion films stained with Hoffman's violet, malachite green, and a mixture of eosine B. and aurantia, and, as will be noticed, are complementary to the three primary colors with which the final print is made. For the negatives produced with the orange-red and the green screens, isochromatic plates are used; that made with the violet screen is non-isochromatic. From these negatives bichromated gelatine printing plates are made, and printed on a lithographic press, the yellow print first, the red superimposed on it, and the blue on the other two.

It is evident that from first to last the registration, or exact coincidence of all parts of the different colored images, must be perfect. As regards the printing, the registration is obtained by the ordinary methods in use for such work, and is not more difficult from photomicrographic negatives than from others. On the other hand, the registration of the magnified images of the object in the camera presents the main difficulty, as the relations and positions of all parts of the installation must remain the same throughout the time necessary to make the three exposures. These results, with the higher powers, are only to be obtained by exercise of the utmost care. The image and the light are first centred and adjusted, the focussing being done through a yellow screen; the yellow screen is removed, and then, in succession, the orange-red, the green, and the violet screens are substituted, and a negative made with each.

M. Du Hauron has recently pointed out in regard to ordinary photography, that some subjects could be rendered quite well enough by two colors. In photomicrography this is a distinct gain, as, frequently, two colors are all that are

in the original; and the gain in time, in lessening the danger of non-registration, and in cost, is of great advantage. The illustration accompanying these notes, of Bacillus Tuberculosis in Sputum at 1000 diameters, stained with carbolic-acid fuchsin and aqueous solution of methylen blue, was produced in this way. Having originally been taken and printed in three colors, it was afterwards, for the purposes of this article, printed from the red and blue printing plates only.



BACILLUS TUBERCULOSIS IN SPUTUM STAINED WITH CARBOLIC ACID FUCHSIN AND AQUEOUS METHYLEN BLUE. X 1000.